

Article

Masquerading predators deceive prey by aggressively mimicking bird droppings in a crab spider

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Abstract

In aggressive mimicry, a predator accesses prey by mimicking the appearance and/or behavior of a harmless or beneficial model in order to avoid being correctly identified by its prey. The crab spider genus *Phrynarachne* is often cited as a textbook example of masquerading as bird droppings (BDs) in order to avoid predation. However, *Phrynarachne* spiders may also aggressively mimic BDs in order to deceive potential prey. To date, there is no experimental evidence to support aggressive mimicry in masquerading crab spiders; therefore, we performed a field survey, a manipulative field experiment, and visual modeling to test this hypothesis using *Phrynarachne ceylonica*. We compared prey-attraction rates among BDs, spiders, and control empty leaves in the field. We found that although all prey combined and agromyzid dipterans, in particular, were attracted to BDs at a higher rate than to spiders, other dipterans and hymenopterans were attracted to BDs at a similar rate as to spiders. Both spiders and BDs attracted insects at a significantly higher rate than did control leaves. As predicted, prey was attracted to experimentally blackened or whitened spiders significantly less frequently than to unmanipulated spiders. Finally, visual modeling suggested that spiders and BDs can be detected by dipterans and hymenopterans against background leaves, but they are indistinguishable from each other. Taken together, our results suggest that insects lured by spiders may misidentify them as BDs, and bird-dropping masquerading may serve as aggressive mimicry in addition to predator avoidance in *P. ceylonica*.

Key words: aggressive mimicry, lure, manipulated spiders, masquerade, prey attraction, *Phrynarachne ceylonica*.

Mimicry can occur when a mimic uses its resemblance to a model to gain a selective benefit by changing a receiver's behavior (Dalziell and Welbergen 2016). Mimicry is a widespread phenomenon across animals, plants and fungi, and mimics may imitate signals or cues in

a wide range of ecological contexts (reviewed in Stevens 2013; Dalziell and Welbergen 2016; Quicke 2017; Ruxton et al. 2018). Typically, mimicry evolves to defend against predators (i.e., defensive or protective mimicry), but mimicry can also evolve in some

predators to lure and capture their prey (i.e., aggressive mimicry). In aggressive mimicry, a predator, parasite (including brood parasite), or parasitoid evolves to mimic signals or cues of a harmless or beneficial model, allowing them to avoid identification by their prey or host (reviewed in Stevens 2013; Jamie 2017). Aggressive mimicry occurs commonly across a wide variety of animals, sensory modalities (e.g., visual, chemical, and vibratory), and contexts (e.g., food source or mate). In some situations, the aggressive mimic imitates a resource that is vital to their prey's survival such as nutrition or a mate. For example, bolas spiders *Mastophora dizydeani* mimic pheromones of female *Spodoptera frugiperda* moths (i.e., mates) in order to lure and capture male moths (Eberhard 1977). Orchid mantises *Hymenopus coronatus* mimic orchid flowers (i.e., food resource) in order to attract vision-guided pollinators (O'hanlon et al. 2014). Aggressive mimicry has many forms and functions (Starrett 1993; Jamie 2017), but some examples, such as masquerading animals, remain poorly understood.

Aggressive mimicry can be used by predators to remain undetected by prey as they approach. The females of some flower-dwelling crab spiders are also known to use aggressive mimicry by resembling to the color of the flowers they are sitting on to become cryptic to flower visitors, such as honeybees (Chittka 2001; Théry and Casas 2002, 2009; Théry et al. 2005; Théry 2007; Defrize et al. 2010). Other flower-dwelling crab spiders, which are highly conspicuous (e.g., UV color contrast against the flower petals) to their prey, choose flowers frequently visited by their prey to attract pollinators (Heiling et al. 2003, 2005; Llandres and Rodríguez-Gironés 2011; Rodríguez-Morales et al. 2018). Whereas these crab spiders depend on flowers to forage, the females of *Epicadus heterogaster* crab spider have conspicuous abdomens that resemble the shape of a flower, and can lure pollinators, not on flowers, but on green leaves, even in the absence of nearby flowers (Vieira et al. 2017). Their abdominal protuberances may reflect UV signals similar to those of flowers, and thus attract pollinators in an aggressive mimicry ploy. However, whether masquerading animals use aggressive mimicry remains unclear.

Masquerading animals have evolved diverse behavioral and/or visual adaptations to closely resemble inedible and often inanimate objects that are commonly found in their native habitats, such as leaves, stones, twigs, and bird droppings (BDs; Cott 1940; Edmunds 1974; Skelhorn 2015; Ruxton et al. 2018). For example, caterpillars of the giant swallowtail *Papilio cressphontes* appear to resemble BDs, larvae of the early thorn moth *Selenia dentaria* masquerade as twigs of the branches they sit on, and dead-leaf butterflies *Kallima inachus* are easily mistaken for dead leaves (Edmunds 1974). Masquerade generally serves as predator avoidance, and increasing evidence supports the protective masquerade hypothesis that predators detect masquerading prey but mistake them for the inedible objects they resemble, and thus ignore them (Skelhorn et al. 2010; Skelhorn 2015). However, masquerade can also be employed by predators, and can serve to hide a predator from its prey or to lure its prey (i.e., the aggressive masquerade hypothesis; Skelhorn 2015, 2018; Quicke 2017). For example, ghost mantises' *Phyllocrania paradoxa* resemblance to dead leaves not only helps them to avoid predation but also to capture prey. In doing so the mantis resembles an inedible or uninteresting object, which prey can detect, but mistakes them for an innocuous item, making them less wary and easier for the predators to catch (Skelhorn 2018). Unlike ghost mantises, in which the model (i.e., a dead leaf) is inedible or innocuous for the receiver (i.e., prey), some animals mimic BDs, which are a food source for many coprophagous insects (Jacobson 1921; Mascord 1980;

Syed and Leal 2009). Thus, mimicking a BD may function in predator avoidance but may also serve as aggressive mimicry to actively lure prey for which BDs are a food source (Quicke 2017).

Masquerading is practised by many spiders (Pekár 2014), but only the protective hypothesis has been experimentally tested to date (Liu et al. 2014; Zhang et al. 2015; Xavier et al. 2018). The sit-and-wait crab spider genus *Phrynarachne* (Araneae, Thomisidae) is an often-cited example of BD masquerade and widely accepted that BD resemblance functions as protective masquerade (Cott 1940; Edmunds 1974; Pekár 2014). It is hypothesized that *Phrynarachne* spiders resemble BDs (see Figure 1A) to also evade recognition and lure insects as prey, because BDs are a food source for some insects such as flies, however, their role as aggressive mimics is yet to be tested (Jacobson 1921; Mascord 1980; Ono 1988; Zhu and Song 2006; Quicke 2017).

We tested the aggressive mimicry hypothesis using the BD spider *Phrynarachne ceylonica* (Figure 1A), in a tropical rainforest. We first observed prey attraction to spiders and compared it to the attraction of both actual BDs and control empty leaves. We predicted that BDs would attract similar numbers and types of prey to spiders if they were indeed aggressively mimicking BDs. We then experimentally blackened or whitened spiders in order to disrupt their mimicry and make them appear less like BDs to test the effect on insect luring. We predicted that color manipulation would increase or reduce the probability of insects being lured by the spiders and would lure different types of insects than would unmanipulated spiders. Our previous study at the same study site (Yu et al. 2015) and the results from this study (see Results section) showed that dipterans and hymenopterans are the most common types of insects attracted to both *P. ceylonica* and BDs. Therefore, we used visual modeling to compare the coloration of *P. ceylonica* and BDs to each other and to background leaves from the perspectives of both dipteran and hymenopteran prey. If *P. ceylonica* spiders lured insects by mimicking BDs as a food source, we predicted that dipterans and hymenopterans would be able to visually differentiate spiders and BDs from background leaves but not from each other, and thus would mistake the spiders as a food source.

Materials and Methods

Field survey of prey attraction

To test the hypothesis that the crab spider *P. ceylonica* aggressively mimics BDs, we conducted a field survey in ca. 250 ha of the Xishuangbanna Tropical Botanic Garden (101°26'948"E, 21°92'123"N, 550 m asl) during the rainy season in 2016 (July–August, average temperature: 22 °C, mean annual rainfall: 1,500 mm). We conducted the survey during the rainy season because spiders and insects are abundant during these months (L. Yu, personal observation). The study took place in a relatively closed-canopy rain forest within an area of ca. 1 ha (500 m long × 20 m wide). *Phrynarachne ceylonica* spiders were commonly (>80%) found on the wide leaves of elephant ear taro *Alocasia macrorrhiza* (Figure 1B), and sometimes on other green broad-leaf plants such as wild elephant foot yam *Amorphophallus paeoniifolius* and *Goniothalamus cheliensis*. Accordingly, we performed our survey and field experiment using exclusively elephant ear taro. This survey provided baseline data on the major groups of insects attracted to spiders and BDs, and facilitated our visual modeling from the perspective of the major prey groups.

To minimize any difference detected among treatments that could arise from spatial or microhabitat-related variation in prey

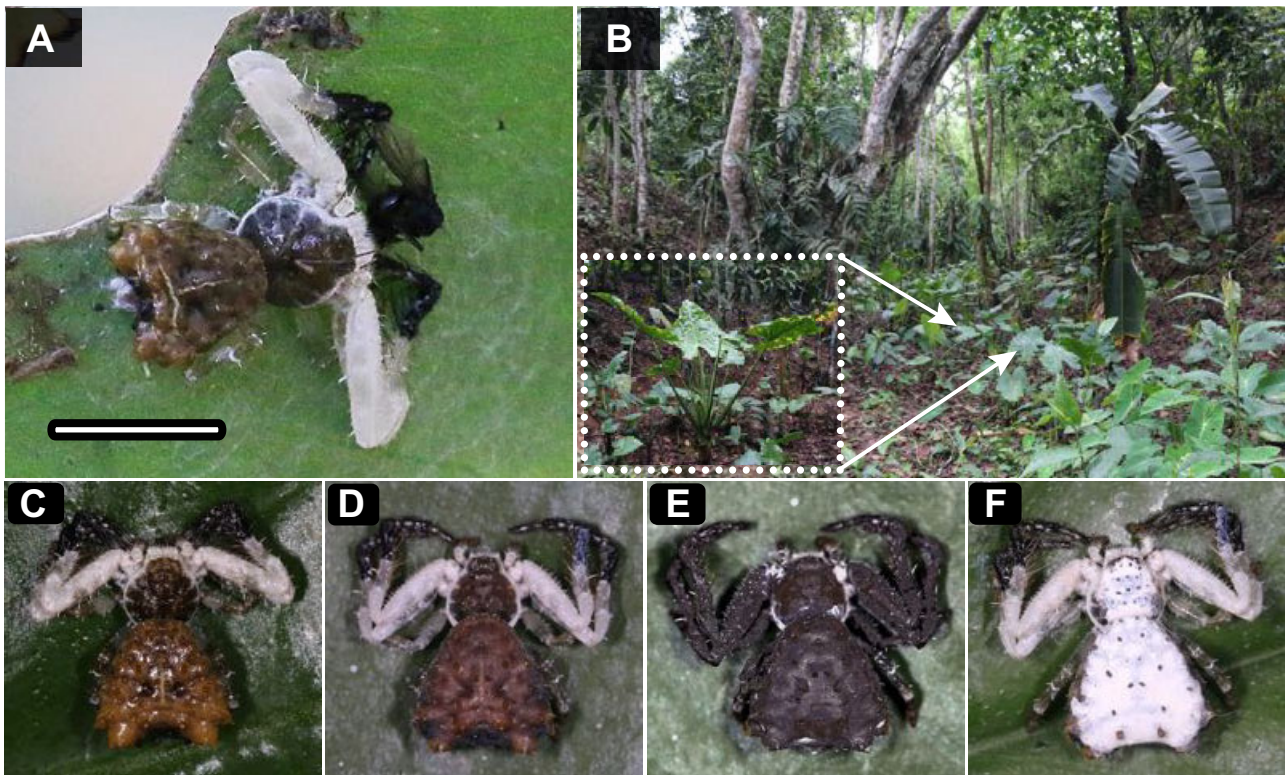


Figure 1. Habitat and 4 different treatment groups of the manipulated and unmanipulated *P. ceylonica* crab spiders used in field experiments. (A) Female *P. ceylonica* on an elephant ear taro *A. macrorrhiza* leaf, eating a dipteran (Agromyzidae). Scale bar = 5 mm; (B) Elephant ear taro *A. macrorrhiza* (inset bottom left) in the rain forest where *P. ceylonica* spiders live and the field experiment was conducted; (C) unmanipulated; (D) sham; (E) blackened; and (F) whitened female *P. ceylonica*.

insect activity, we randomly located 33 spiders ranging from fifth-instar juveniles to adult females (body length: range from 4.3 to 9.1 mm; mean \pm SE = 6.8 ± 0.2 mm), 8 semi-fresh (considerably wet; dry samples were not used) BDs, and 11 bare elephant ear taro leaves as controls. Although there were many BDs at the study site, many of them were either too dry or much larger than the average size of the spiders, which could affect insect visitations to BDs. Our relative small sample of empty elephant ear taro leaves for the control group was due to the availability of a limited number of video cameras, as we attempted to maximize the number of spiders we could observe. Each spider or BD was randomly positioned on an individual leaf, and was separated at least 1 m from each other. Eleven leaves were left bare as control stimuli, and each separated at least 1 m from each other and also from spiders or BDs. Before each trial, we measured the spiders' body length and the BD length. We also attempted to ensure that leaves were positioned at similar heights from the ground. We marked each spider and BD using a waterproof label with a unique code secured to the stem of the plant.

We made video recordings of insect visitation to the spiders, BDs, and empty leaves at each location for 1 h either in the morning between 1,000 and 1,200 (i.e., am session) or in the afternoon between 1,300 and 1,600 (i.e., pm session) in order to record a wide range of insects active at different times of the day. We recorded using video cameras (HDR-PJ600E, SONY, Japan) only in sunny or clouded conditions. We placed cameras \sim 1 m away from each target spider, BD or empty leaf, and at least 10 m apart from each other, allowing us to record several locations simultaneously. We recorded each location for only 1 h per day to ensure that the droppings would not dry out (as insects only consume soft/fresh droppings; L.

Yu, personal observation), and recorded each BD for only a single 1-h period. After all trials were completed, we collected all available spiders for spectral reflectance measurements in the laboratory. For each control leaf, we randomly chose an area on the edge of the leaf similar in size to the average spider size (\sim 10 mm in diameter) and recorded insect visits within this area for 1 h. We chose the circular areas on the edges of leaves because spiders were often found near the edges of leaves.

We examined the video footage to retrieve data on insect attraction to spiders, BDs or empty control leaves. We analyzed data from 25 spiders, each with 2–5 days of \geq 1 h long recordings. Eight spiders were excluded from the data analyses because we collected their data on only 1 day, or their video footage was $<$ 1 h long. In total, we analyzed 100 h of spider, 8 h of BD, and 11 h of control leaf footage. We considered all insects that had landed on leaves as potential prey. Insects that simply scanned spiders/BDs/leaves without landing were considered nonprey (Dafni and Kevan 1996) and were excluded from subsequent analyses. When a landing insect approached a spider or BD to within \leq 1 cm, or landed on the randomly selected area on the empty control leaves, we considered it a visit event. We also recorded the types of insect that visited and classified visitors into orders or families, as described by Yu et al. (2015). We excluded insects that could not confidently be identified from the analyses.

To compare the attraction rates of prey to the spiders, BDs, and empty control leaves, we calculated the per-hour rate of insect attraction (the number of insects attracted per hour) for each spider, BD, and control leaf. We verified the normality and homogeneity of variance of the data by visual inspection of quantile–quantile and

residuals versus fitted values plots as well as using Shapiro–Wilk test and Levene tests, respectively. To test the effects of the treatment (spider, BD, and control leaf) on per-hour rate of total insect attraction, and on each of 3 types of insects, we then ran 4 separate negative binomial generalized linear models with log-link using the package MASS in R version 4.0.5 (R Core Team 2021). Negative binomial generalized linear models were chosen because the data were over-dispersed. We then used the likelihood ratio tests (LRTs) to compare the model that included the treatment as a predictor variable to a null model. After we detected a significant effect of the treatment on the rate of insect attraction, we used post-hoc pairwise comparisons to assess whether the attraction rate differed between the treatments. Holm adjustments were made to significance levels.

Manipulation of spider color pattern

To test whether naturally colored spiders attract more prey than entirely black or white spiders (which would appear less similar to a BD), we conducted a field experiment by manipulating the color pattern of *P. ceylonica* spiders in the same forest during the rainy season as our observational study. Our intent was to make the spiders appear less similar to BDs, and to create a uniform resemblance to droppings by blackening and whitening spiders, although we note that the proportion of black and white patches in BDs varies in nature. We expected that the color-manipulated spiders would attract insects more or less frequently than unmanipulated spiders, and would attract different types of insects. Before the experiment, we collected 31 spiders at ages ranging from fifth-instar juveniles to subadult females (body length, range: 3.4–7.2 mm; mean \pm SE = 4.9 \pm 0.2 mm). Before each trial, we chose 4 similarly sized spiders, and assigned them randomly to one of 4 treatment groups unmanipulated, sham control, blackened, or whitened (Figure 1C–F). We manipulated the spiders' appearance using non-toxic and odorless water color paints (Deli Co Ltd, China), which had little to no adverse effects on spider behavior; spiders showed normal locomotion when disturbed and consumed fruit flies when offered (L. Yu, personal observation). We chose colors with similar reflectance properties to the dark and light patches on spiders (see Results section). We did not alter the coloration of spiders in the first group (unmanipulated spiders). In the second group (sham controls), we painted the dorsal side of the abdomen, the dorsal side of the carapace, and the femurs of the first pair of legs the same color of those areas in unmanipulated spiders. This accounted for any potential effects of chemical cues emitted from the paints without changing the color pattern of the spiders. The third group (blackened spiders) was painted entirely black (using a mixture of “black” and “brown” at a ratio of 3:1), and the 4th group (whitened spiders) was painted entirely white. We painted the spiders ~30 min before trials. Because of the limited numbers of spiders in the field ($n = 31$), we tested individual spiders up to 3 times, but they were painted different patterns in different treatments. When spiders were retested, we removed their previous paint by applying a drop of water to the painted region for ~1 min and gently clearing the paint with a small brush, before painting them with a new color as described above.

We did not analyze the commonly used interception or attraction rate of prey (i.e., the number of insects that was intercepted or attracted per hour, Tso et al. 2004; Chuang et al. 2008; Tan et al. 2010) because once a crab spider is satiated it is unlikely to consume additional prey items, and 1 prey item may be sufficient for the spider. Thus, we carried out a 4-way prey choice experiment instead, by randomly placing spiders from each of the 4 treatment groups on the 4 corners of the same leaf (maximal leaf length: ca. 50 cm), at

least 10 cm apart from each other. Like typical crab spiders (Morse 2007), *P. ceylonica* are sit-and-wait predators, and usually remain stationary for a considerable time (>12 h during the daytime; L. Yu, personal observation). We allowed all 4 spiders to acclimate for 10 min on the leaf, and then recorded the insects with video cameras mounted at least 2 m away from the leaf for 1 h between 1,000 and 1,600 h. This allowed us to observe which of the 4 spiders attracted an insect first—defined as which of the 4 spiders was approached when the first insect landed on the leaf. The trial ended when an insect first approached a spider or when 1 h elapsed, whichever came first. After each trial, we returned the spiders to their holding containers (diameter \times height: 5 \times 8 cm) for additional testing on a different leaf at a different time of day. In order to maintain independence of the trials and control for potential effects of chemical cues left on the leaves, no leaves were used more than once. We conducted 80 4-choice trials.

Using the video footage, we counted the number of spiders in each treatment group that first attracted an insect. We also noted the type of insect (a dipteran [agromyzid or other dipterans], hymenopteran, or other insects) that was first attracted to the spider in each treatment group. Four trials were excluded from analysis because none of the spiders attracted any insects.

We used a Kruskal–Wallis test to compare body length among the 4 spider treatments because the data were not normally distributed. To test for effects of color manipulation on the frequency of spiders that attracted the first insect, we performed an exact multinomial test for a goodness of fit (null hypothesis: the probability of an insect visiting any of the 4 choices is the same, i.e., 25% for each choice) followed by post hoc pairwise comparisons with an *fd*r correction for the adjusted *P*-value, using the R package *RVAideMemoire* version 0.9-79 (Hervé 2021). We also used a chi-square (χ^2) test to test for differences in the composition of prey among the treatment groups.

Spectrophotometric measurement

We measured the spectral reflectance of semi-fresh BDs ($n = 10$), female spiders ($n = 10$), and elephant ear taro leaves ($n = 10$) using an Ocean Optics USB4000 spectrometer with a DH-2000 deuterium and tungsten halogen light source (Ocean Optics Inc., Dunedin, FL, USA) in the laboratory. We followed spectrometric procedures described in other studies (Lim and Li 2006; Zhang et al. 2015), and only the essential details are given here. BDs were collected from light-vented bulbuls *Pycnonotus sinensis* that were commonly found at the study site. We measured the spectral reflectance of 2 contrasting patches (black and white) of each BD and of 3 body parts (“dorsal abdomen,” “dorsal carapace,” and “leg 1 femur”) for each spider. We randomly selected 5 spots within each color patch or body part and recorded the reflectance reading (300–700 nm) perpendicular to and 2 mm above the sample. For incorporation into physiological visual models (see below), we also measured the spectral reflectance of the background leaves (elephant ear taro *A. macrorrhiza*) using the same method as above ($n = 10$, 50 readings). We then averaged these 50 readings and used the mean as the background reflectance spectrum in visual modeling.

For the color-pattern manipulation experiment, we also measured the reflectance spectra of spiders with a blackened dorsal abdomen ($n = 9$), a whitened dorsal abdomen ($n = 9$), and a dorsal abdomen painted the same color as a natural dorsal abdomen (i.e., sham control; $n = 9$). Doing so allowed us to assess whether the coloration (white or black) of the painted spider bodies was similar to the coloration (white or black) of the unmanipulated

spider bodies when viewed from the perspectives of dipteran and hymenopteran prey.

Visual modeling from the perspectives of dipteran and Hymenopteran prey

To distinguish among crypsis, prey attraction, and aggressive mimicry as explanations for BD masquerading, it is crucial to assess the resemblance between spiders and BDs and their visibility against the background leaves. If spiders are undetectable to an insect when viewed against the background leaves, then the crypsis hypothesis can be used to explain the results. However, if spiders and BDs are indistinguishable from insect prey, spiders may be using their visual resemblance to BDs to lure insects. Therefore, we quantified the color contrasts between spiders and BDs against the background leaves on which they were presented. We also quantified the color contrasts between spiders and BDs when viewed by Diptera and Hymenoptera, as their sensitivities best represent the spiders' potential prey.

Visual modeling includes the signal reflectance, the visual system of the viewers, the background reflectance, and the ambient light spectrum. Spectral reflectance as measured above was used to model the visual systems of dipteran and hymenopteran prey and to calculate the color contrast perceived by them. We used the illumination standard D65 (CIE) as our ambient light spectrum in all analyses. We assumed that all visual interactions happened under sunny daylight conditions (Defrize et al. 2010), as both our field survey and manipulative experiment were mostly conducted on sunny days. We performed all spectral processing, analyses, and visual modeling using the R package *pavo* version 2.4.0 (Maia et al. 2019).

We calculated color contrasts between spiders and BDs, and between spiders or BDs and the background leaves using the color visual model proposed by Chittka (1992) following earlier studies (Théry and Casas 2002; Liu et al. 2014; White et al. 2017; Ximenes and Gawryszewski 2019), and only essential details are given here. To model the chromatic channel of dipteran and hymenopteran vision, we used the photoreceptor sensitivities of the fruit fly, *Drosophila melanogaster* (Schmaitmann et al. 2013) and the honeybee, *Apis mellifera* (Hempel de Ibarra et al. 2014; Menzel and Backhaus 1991), respectively. As fruit flies and honeybees have tetra and trichromatic vision, respectively, we calculated the Euclidean distance (ΔS) between 2 points in color space (E_{UV} , E_{BLUE} , and E_{GREEN} for honeybees, E_{UV} , E_{BLUE} , E_{GREEN} , and E_{RED} for fruit flies) following the procedures described in previous studies (Chittka and Kevan 2005; Defrize et al. 2010; White et al. 2017; Xavier et al. 2018; Ximenes and Gawryszewski 2019). We used one-sample *t*-tests to evaluate whether the chromatic contrast values (i.e., mean Euclidean distance) for each spider and BD, each spider and leaf, or each BD and leaf were significantly greater than the dipteran or hymenopteran prey detection threshold of 0.11 and 0.045 hexagon units, respectively (Théry and Casas 2002; Dyer and Chittka 2004; Dyer and Neumeyer 2005).

Honeybees are known to use only green photoreceptors to detect achromatic targets at long ranges (Giurfa et al. 1997; Spaethe et al. 2001), whereas fruit flies use only the outer photoreceptors (R1–R6; Kelber and Henze 2013), to detect achromatic targets at long ranges. From the perspective of a hymenopteran, we calculated achromatic contrast (at a long distance) between spiders and BDs by dividing the spider's E_{GREEN} by the BD's E_{GREEN} , and between spiders and leaves by dividing the spider's E_{GREEN} by the leaf's E_{GREEN} (Théry and Casas 2002). We calculated achromatic contrasts from viewing fly prey in the same way as for hymenopteran prey, except that E_{R1-6} was used. We then used one-sample *t*-tests to test whether

achromatic contrasts of either spiders and BDs with respect to their background leaves, or spiders with respect to BDs were significantly >1.0 , as predicted for equal brightness (Théry and Casas 2002).

Results

Field survey

During field observations, spiders and BDs attracted dipterans (primarily agromyzids), hymenopterans, lepidopterans, and other insects. Eight BDs attracted 481 insects, 88.3% of which were dipterans (primarily agromyzids), 7.5% were hymenopterans, and 4.2% were other insects (Figure 2A). *Phrynarachne ceylonica* spiders ($n=25$) attracted 751 insects, 85.4% of which were dipterans (also primarily agromyzids), 13.3% were hymenopterans and 1.2% were other insects (Figure 2B). Eleven empty control leaves were visited by only 26 insects, 42.3% of which were dipterans, 15.4% were hymenopterans, and 42.3% were other insects (Figure 2C).

Treatment (spider, BD, and control leaf) had a significant effect on the attraction rate for all insects combined (LRT: $\chi^2=57.18$, $df=2$, $P<0.0001$), agromyzids ($\chi^2=63.51$, $df=2$, $P<0.0001$), other dipterans ($\chi^2=29.78$, $df=2$, $P<0.0001$), and hymenopterans ($\chi^2=19.71$, $df=2$, $P<0.0001$). BDs showed a higher per-hour attraction rate for insects combined and for agromyzids than did spiders and empty controls, but spiders and BD attracted other dipterans at similar rates. Spiders attracted insects combined, agromyzids, and other dipterans at a significantly higher rate than did empty controls. However, the per-hour rate of hymenopteran attraction was not significantly influenced by the treatment (Figure 2D, E).

Manipulation of spider color pattern

There was no significant difference in body length among spiders in the 4 treatments (mean \pm SE; original: 5.1 ± 0.9 mm, sham: 4.9 ± 0.8 mm, blackened: 4.7 ± 0.5 mm, whitened: 4.8 ± 0.7 mm; Kruskal–Wallis test: $\chi^2=5.16$, $P=0.161$). This suggests that spider body size may not disproportionately affect the rate of insect attraction among treatments. However, manipulating the appearance of spiders significantly affected their probability of attracting the first insect (Exact multinomial test: $P=0.0006$, $N=76$). Results from post-hoc pairwise comparisons showed that insects were first attracted to the manipulated (i.e., blackened or whitened) spiders significantly less frequently than to the unmanipulated spiders (Figure 3A). There was no significant difference in the frequency of the insects attracted to the original versus sham control spiders. The frequency of insects attracted to the sham control spiders was also not significantly different from the frequency of the insects attracted to the whitened spiders (Figure 3A). Lastly, there were no significant differences in the composition of insects that were attracted to the spiders among the 4 treatment groups (χ^2 test for independence: $\chi^2=5.039$, $df=9$, $P=0.831$, $N=76$; Figure 3B).

Dipteran and hymenopteran visual modeling

Phrynarachne ceylonica and BDs had similar spectral reflectance to each other for both their dark and their white patches (Figure 4). Results from color contrasts showed that both chromatic and achromatic contrast values were significantly lower than or not significantly different from the chromatic contrast threshold for both dipterans and hymenopterans except for the achromatic contrast between the spider carapace and the dark patch on BDs (Figures 5 and 6). This suggests that both dipteran and hymenopteran prey could not in theory distinguish spiders from BDs chromatically (from a short distance) and

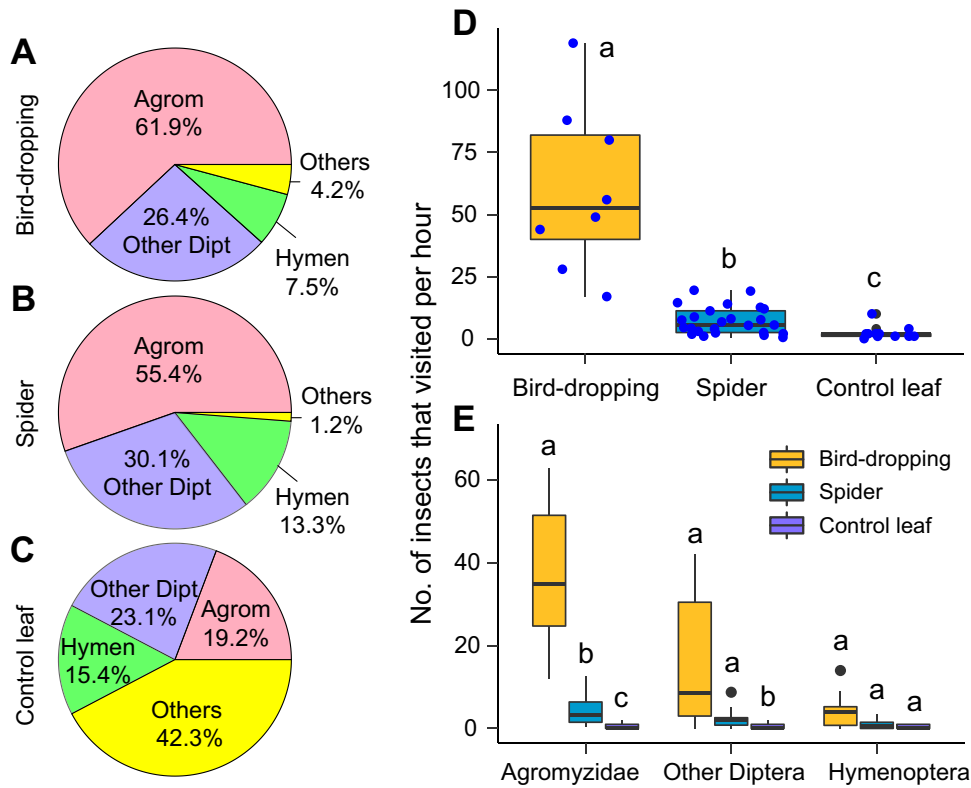


Figure 2. Composition of insects that visited (A) BDs ($n=8$), (B) *P. ceylonica* crab spiders ($n=25$), and (C) control empty leaves ($n=11$) during the rainy season in the Xishuangbanna Tropical Botanic Garden. Agrom = Agromyzidae, Other Dipt = Other Diptera, Hymen = Hymenoptera, Others = other insects (Coleoptera, Lepidoptera, and Orthoptera). (D) Boxplot shows the per-hour attraction rate of insects combined by spiders, BDs, and empty control leaves. (E) Boxplot showed the per-hour attraction rate of agromyzid dipterans, other dipterans, and hymenopterans by spiders, BDs, and empty control leaves. Boxplots show the median (central line), first and third quartiles (box), and minimum and maximum values (whiskers). Different letters above the bars indicate significant differences between the treatments.

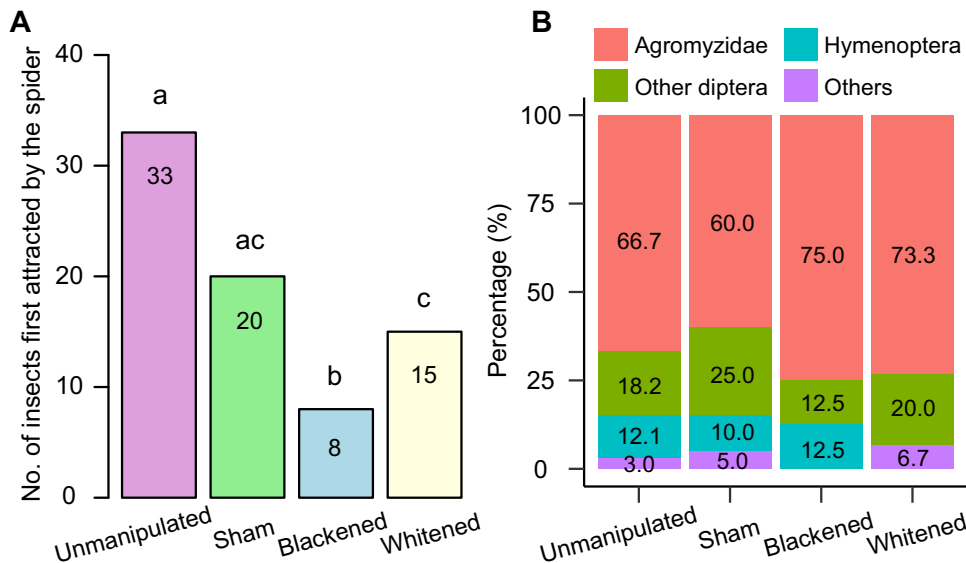


Figure 3. (A) Number of insects that were first attracted to *P. ceylonica* spiders in the 4 different treatments (unmanipulated, sham control, blackened, and whitened); and (B) the percentages (%) of each major type of insect prey that were first attracted to the spiders among the 4 treatments of spiders. Different letters above the bars indicate statistical difference.

achromatically (from a long distance). Whereas the chromatic contrast values between spiders or BDs and background leaves were not significantly different from or significantly lower than the threshold for

dipterans, the achromatic contrast values between spiders or BDs and background leaves were significantly higher than the achromatic contrast threshold for dipterans except for a spider's dark carapace

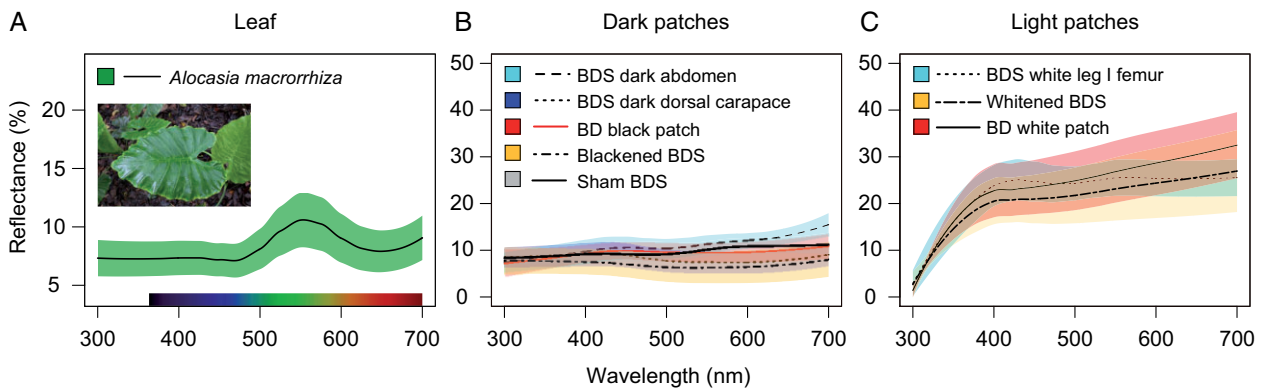


Figure 4. Mean (\pm SD) spectral reflectance of the background leaves (A), the dark (B), and the white (C) patches of manipulated (blackened and whitened), unmanipulated, and sham control *P. ceylonica* crab spiders bird-dropping spiders (BDS), as well as BD dark and white patches. Inset (first panel) shows the leaf used in the field experiment.

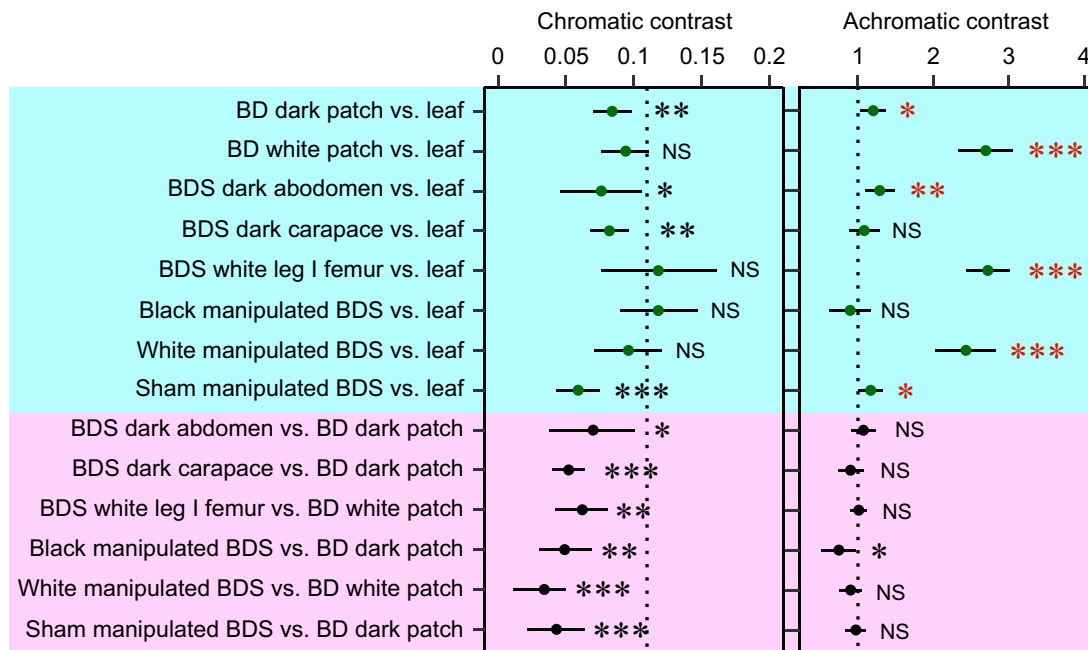


Figure 5. Mean with 95% confidence interval chromatic and achromatic contrast values for spiders (unmanipulated and manipulated *P. ceylonica* spiders [BDS]) when viewed by dipteran prey against background leaves or BDs. The dashed lines at 0.11 and 1.00 represent the chromatic and achromatic contrast thresholds for hymenopterans, respectively. The red and black asterisks represent a significantly higher or lower color contrast value than the contrast threshold for hymenopterans, respectively, based on the results from one-sample *t*-tests. NS indicates no significant difference.

(Figure 5). This suggests that dipteran prey could not chromatically detect spiders or BDs on the background leaves, but they could achromatically detect them on the background leaves. However, the color contrasts of hymenopteran vision (Figure 6) showed that hymenopterans could in theory detect both spiders and BDs on the background leaves from both short (chromatically) and long distances (achromatically) except for the achromatic contrasts of the dark patch on a spider’s carapace and a BD’s dark patch.

Discussion

Our experimental, field survey, and visual modeling results showed that mimicking BDs may cause insect prey species to misidentify predatory *P. ceylonica* spiders as BDs. This allows us to rule out

crypsis as the explanation for these spiders luring prey. The misidentification is probably explained by the indistinguishability between the colors of spider bodies and BDs when viewed by dipteran and hymenopteran visual systems. Thus, our results support the hypothesis that the crab spider *P. ceylonica*, a mimic of BDs, uses masquerade as an aggressive mimicry ploy.

In the crab spider *P. ceylonica*, BD masquerade may not only function to avoid predation (Cott 1940; Edmunds 1974; Pekár 2014; Quicke 2017) but may also allow spiders access to prey through aggressive mimicry. We tested this hypothesis by first performing field observations to assess whether *P. ceylonica* would attract similar types and comparable proportions of prey as BDs would in the wild. In turn, our experimental manipulation tested specifically whether the visual appearance of a spider (and its

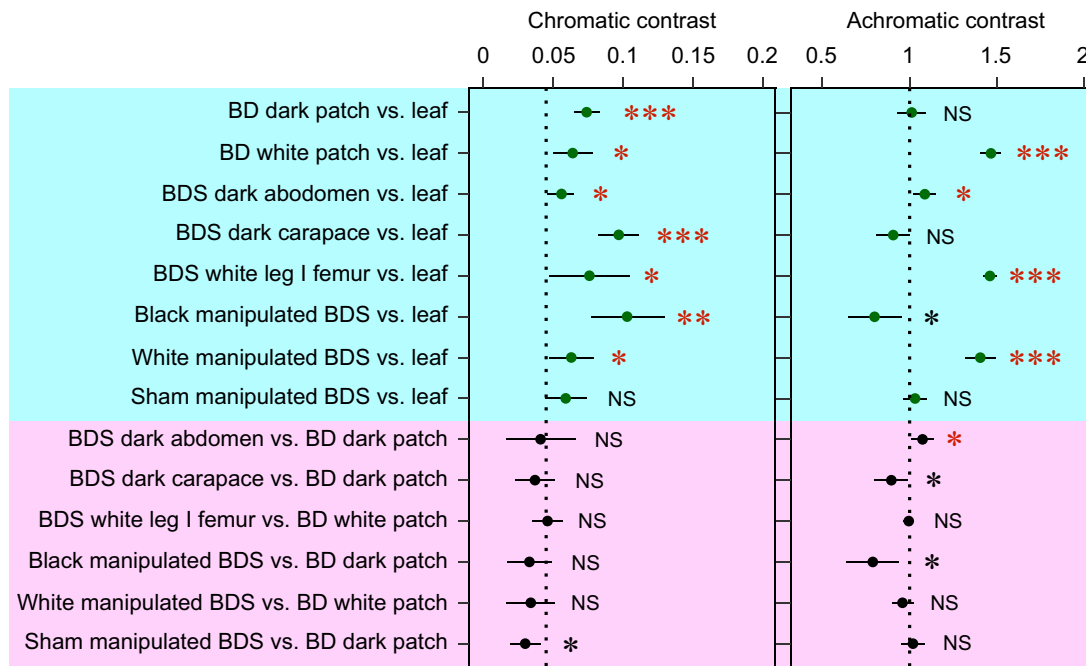


Figure 6. Mean with 95% confidence interval chromatic and achromatic contrast values for spiders (unmanipulated and manipulated *P. ceylonica* spiders [BDS]) when viewed by hymenopteran prey against background leaves or BDs. The dashed lines at 0.045 and 1.000 hexagon units represent the chromatic and achromatic contrast thresholds for hymenopterans, respectively. The red and black asterisks represent a significantly higher or lower color contrast value than the contrast threshold for hymenopterans, respectively, based on the results from one-sample *t*-tests.

resemblance to BDs) affects insect attraction. As predicted, the types of prey attracted to the spiders were similar to those attracted to BDs in the same habitat (Figure 2). The most common prey species attracted to both spiders and BDs were dipterans, particularly agromyzid flies (Figure 2; Jacobson 1921; Mascord 1980; Prokopy et al. 1993; Ibáñez-Álamo et al. 2016). Like other flies, agromyzids have good eyesight (Land 1997; Land and Nilsson 2012), and these flies likely rely on visual cues to locate food although chemical cues may also play a role. However, further study is needed to confirm whether agromyzid flies are *P. ceylonica* spiders' main prey.

The finding that *P. ceylonica* spiders lure prey insects by mimicking BDs is supported by our visual modeling data and manipulative experimental results. Dipteran and hymenopteran prey can detect a *P. ceylonica* spider against a background leaf, but appears to be unable to perceive them as different (Figures 5 and 6). More importantly, our experimental results support our prediction that spiders that looked less similar to BDs (i.e., blackened or whitened) were less attractive to insects, although the types of insects attracted to manipulated spiders were similar to those attracted to the unmanipulated spiders (Figure 3). There was a reduction in the frequency of prey attraction to the sham control spiders (Figure 3A), which was not significantly different from frequency of prey attracted to spiders in the whitened treatment group. This is probably an effect of paint per se on prey behavior, and we should be cautious about the complication implied by the sham control. Nevertheless, our visual modeling data showed that a dipteran or a hymenopteran prey may not perceive the differences between a *P. ceylonica* spider and a BD, and this may confer selective benefits onto *P. ceylonica* spiders. Thus, *P. ceylonica* spiders may have evolved BD masquerading to lure insect prey by using aggressive mimicry. Because the BD masquerade of *P. ceylonica* may help this spider both avoid predators and lure prey, it is of interest to investigate how the selective pressures of predators and prey interact to affect *P. ceylonica* phenotypes.

Other masquerading predators do not use aggressive mimicry to attract their prey, but instead use aggressive mimicry for closer access to their victims before attacking. For example, aggressive masquerade in the dead-leaf-resembling ghost mantis is a different form of aggressive mimicry in which the model (Skelhorn et al. 2010; Skelhorn 2015, 2018), a dead leaf, is an innocuous object that incurs no fitness costs or benefits to prey (Jamie 2017). Prey crickets mistake the mantises for dead leaves, and are not wary of them, and are therefore easier for the mantises to catch. This form of aggressive mimicry may allow the masquerading predator to more closely approach the prey, or may simply prevent the prey from actively avoiding the predator. Aggressive mimicry in *P. ceylonica* spiders resembles that of ghost mantises, but with a critical difference that the BDs that *P. ceylonica* spiders resemble, unlike the dead leaves, are a food source for the target animals. Therefore, masquerading predators can use different aggressive mimicry ploys depending on whether the model is innocuous or a food source.

In addition to visual cues, scent is likely to be important for the ability of dipterans and hymenopterans to detect BDs (Syed and Leal 2009). *Phrynarachne ceylonica* may resemble BDs not just visually but also chemically to attract prey, as evidence shows that these spiders smell like BDs (Jacobson 1921; Mascord 1980; Ono 1988; Gray 1991; Zhu and Song 2006). Our field survey showed that BDs attracted insects combined (primarily dipterans) at a significantly higher rate than spiders (Figure 2B). This suggests that prey insects may be more attracted to the odor of BDs. In addition, flies have been observed to swarm around the spiders (Jacobson 1921; Yu et al. 2015). BDs were, however, by far the most attractive stimulus to prey in our field survey, and BDs may emit a stronger odor than do BD mimicking spiders. It is therefore of interest to investigate whether *P. ceylonica* spiders also release a chemical that aggressively mimics the smell of BDs, and how this cue may interact with visual

aggressive mimicry to cause prey insects to misidentify the predatory spiders.

In summary, our study suggests that *P. ceylonica* benefits from its apparent BD masquerade and lures prey via aggressive mimicry. Although the proximate mechanisms by which prey mistake *P. ceylonica* spiders for BDs remain unclear, our study shows that a wide variety of dipterans and hymenopterans are able to detect *P. ceylonica* spiders, but are still lured by them in large numbers. The spiders' visual resemblance to BDs is the most likely mechanism for this. We thus conclude that the appearance of *P. ceylonica* represents aggressive mimicry of BDs and aids in effective predation. Overall, our study not only provides evidence supporting the aggressive mimicry hypothesis for this species of crab spider but also sheds light on how common aggressive mimicry is among animals.

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Authors' Contributions

D.L., L.Y., X.X., C.J.P., and X.D.Y. conceived and designed the study. L.Y., X.X., and Z.T.Z. collected the data. D.L., L.Y., and C.J.P. analyzed the data. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of Interest Statement

We declare we have no competing interests related to this work.

Data Accessibility Statement

All data and R codes used in this study are available in the Mendeley Data (doi:10.17632/hkvz75gg82.1).

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